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Role of physical exercise on hepatic insulin, glucocorticoid and inflammatory signaling pathways in an animal model of non-alcoholic steatohepatitis

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ABSTRACT

Aims: Pro-inflammatory mediators, glucocorticoids and transforming growth factor (TGF)-β are implicated in the pathogenesis of non-alcoholic steatohepatitis (NASH)-related insulin resistance. As physical activity is beneficial against NASH, we analyzed the voluntary physical activity (VPA) and endurance training (ET) (preventive and therapeutic strategies) effects on hepatic insulin, pro-inflammatory and glucocorticoid signaling regulators/mediators in high-fat (Lieber-DeCarli) diet (HFD)-induced NASH.
Main methods: Adult male Sprague–Dawley rats were divided in standard diet (SD) or HFD, with sedentary, VPA and ET animals in both diet regimens. Plasma glucose and insulin concentrations were analyzed; plasma insulin sensitivity index (ISI) was calculated. Hepatic insulin, pro-inflammatory and glucocorticoid signaling regulators/mediators were evaluated by Western blot or reverse transcriptase-PCR.
Key findings: ET improved ISI in both diet regimens. HFD-feeding increased interleukin-1β and induced a similar pattern on interleukin-6 and TGF-β, which were globally reduced by physical exercise. ET decreased HFD leukemia inhibitory factor level, SD + VPA animals presenting higher values than HFD + VPA animals. HFD increased the ratio of IRS-1^{Ser307}/total IRS-1, which was completely mitigated by physical exercise. Physical exercise reduced total ERK and JNK (total and activated) expression in HFD. In SD *vs.* HFD, VPA presented higher activated JNK and ET presented higher total JNK. Generally, in HFD, the ratio (activated/total) of AKT, and each separately, decreased with exercise and also for activated AKT in SD. Overall, in both diets, exercise reduced 11β-hydroxysteroid dehydrogenase type 1. ET increased glucocorticoid receptor and reduced PTP1B in HFD.
Significance: Physical exercise mitigates the expression of pro-inflammatory mediators and positively modulates insulin and glucocorticoid signaling in NASH.

Introduction

The prevalence of NASH, a progressive form of non-alcoholic fatty liver disease (NAFLD), increased dramatically in the last few years as a consequence of excessive consumption of high-caloric food and/or sedentary life style [1,2]. NASH is characterized, among other factors, by aberrant hepatic lipid droplet accumulation, pro-inflammatory cellular environment and insulin resistance [1–4].

Although the precise mechanisms underlying NASH-related hepatic insulin resistance are not yet completely understood, several studies implicate hepatic inflammation and ectopic deposition of fat as well as hepatic increased levels of glucocorticoids and increased TGF-β signaling in the process [3–8].

Hepatic inflammation, mediated by nuclear factor κ-light-chain-enhancer of activated B cells (NF-κB), NF-κB inhibitor (IκB) kinase (IKK) and/or c-Jun N-terminal kinase (JNK) pathways, coupled with hepatic ectopic lipid deposition (coursing with diacylglycerol and ceramide accumulation), induces and/or exacerbates hepatic insulin resistance [5,6,9–12]. Additionally, increased protein tyrosine phosphatase 1B (PTP1B, the major regulator of the insulin signaling pathway) expression has been observed in liver biopsies of NASH patients [13] as well as in the liver of HFD-fed mice [14], which can be linked to hepatic insulin resistance [14–18] and inflammation, since tumor necrosis

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factor alpha (TNF-α) induces both mRNA and protein expression of hepatic PTP1B via nuclear NF-κB activation [14]. Both PTP1B and 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) are abundant enzymes located/tethered on the cytosolic surface of the endoplasmic reticulum (ER) [17].

11β-HSD1 regulates tissue glucocorticoid action, at the pre-receptor level, through the conversion of inert cortisone into biologically active cortisol in humans (or dehydrocorticosterone into corticosterone in rodents). Glucocorticoid receptor (GR) and sirtuin 1 (Sirt1) are important in glucocorticoid signaling [3,7,19–21]. Increased glucocorticoid signaling is associated with insulin resistance [7,20,22]. Additionally, hepatic mRNA expression and/or activity of 11β-HSD1 and GR parallels NASH progression, which implies a role in response to chronic inflammation and suggests that glucocorticoid metabolism is critical in the onset and/or progression of NASH [3]. Likewise, increased hepatic TGF-β signaling is closely associated with hepatic insulin resistance and contributes to hepatic steatosis, inflammation, fibrosis and hepatocyte death [4,8,23,24], suggesting TGF-β as a relevant contributor to the progression of NASH [25].

Several epidemiological and observational studies suggest that physical inactivity is associated with obesity, systemic inflammation, hepatic insulin resistance and type 2 diabetes mellitus [26,27]. On the other hand, regular exercise training is widely accepted as a non-pharmacological tool against isolated features included in NASH, such as hepatic steatosis and hepatic inflammation, in both humans and rodents. Additionally, regular exercise training improves insulin sensitivity in the liver and glucose homeostasis [26,28,29]. However, the impact of physical exercise on the specific context of NASH-related hepatic insulin, glucocorticoid and pro-inflammatory signaling pathways is undetermined so far. Therefore, the objective of this work was to analyze the effects of VPA and ET (preventive and therapeutic strategies) on hepatic insulin, pro-inflammatory and glucocorticoid signaling regulators/mediators in HFD (Lieber-DeCarli)-induced NASH. Here, in an animal model of HFD-induced NASH, we found that both VPA and ET had positive effects on those signaling pathways.

Materials and methods

Treatment of animals

Thirty-six male Sprague–Dawley rats (aged 5–6 weeks and weighing 125–150 g) were purchased from Charles River (L'Arbresle, France). Animals were individually housed in cages (with an enriched environment) and maintained in a temperature and humidity-controlled room (21–22 °C; 50–60% humidity) with reversed 12 h light/dark cycles, with *ad libitum* access to water and food (provided in the liquid state).

As shown in Fig. 1, the feeding and activity protocols were preceded by a 1-week period for adaptation to the liquid diet, in which standard control liquid diet was given to all animals. Then, the animals were randomly ascribed into six groups as follows: standard liquid diet + sedentary animals (SS), standard liquid diet + voluntarily physically active animals (SVPA), standard liquid diet + endurance trained animals (SET), liquid HFD + sedentary animals (HS), liquid HFD + voluntarily physically active animals (HVPA) and liquid HFD + endurance trained animals (HET) [30]. The liquid HFD [previously described to induce NASH [31] provided 71% of energy from fat, 11% from carbohydrate and 18% from protein (Lieber-DeCarli diet #712031) and the isocaloric standard control liquid diet provided 35% of energy from fat, 47% from carbohydrate and 18% from protein (Lieber-DeCarli diet #710027); both diets were purchased from Dyets Inc. (Bethlehem, PA, USA). The study was approved by local Institutional Ethics Committee and followed the guidelines for the care and use of laboratory animals in research advised by the Federation of European Laboratory Animal Science Associations (FELASA) and Portuguese Act 129/92. Several authors of this manuscript are accredited by FELASA to perform animal experimentation.

Animals of the SVPA and HVPA groups had free access to a free wheel throughout the 17 weeks of the protocol and the running distance was obtained daily from a digital counter between 08.00 and 10.00 am. After 8 weeks of diet consumption, half of the SS and HS animals were submitted to endurance training (SET and HET, respectively) while the other half continued to be sedentary. Initially, SET and HET rats were progressively acclimated to the motor driven treadmill for 5 days/week at 15 m/min and 0% grade until 30 min of running was achieved. One-week acclimation was followed by 8 weeks of endurance exercise for 5 days/week, 60 min/day at a starting speed of 15 m/min, which was gradually increased over the training program until 25 m/min was reached. Sedentary animals were placed on a non-moving treadmill 5 days/week for 60 min in order to expose the sedentary animals (SS and HS) to the same environmental conditions but without promoting any physical training adaptation.

Sacrifice, blood samples and liver extraction of animals

Rats were deeply anesthetized with a ketamine/xylazine combination [90 mg/kg of ketamine (Merial, Lyon, France) and 10 mg/kg of xylazine (Bayer, Lisbon, Portugal)]. Blood was collected from the left ventricle and plasma was separated (5000 *g* for 5 min at 4 °C), split into aliquots and stored at −80 °C for later biochemical analyses.

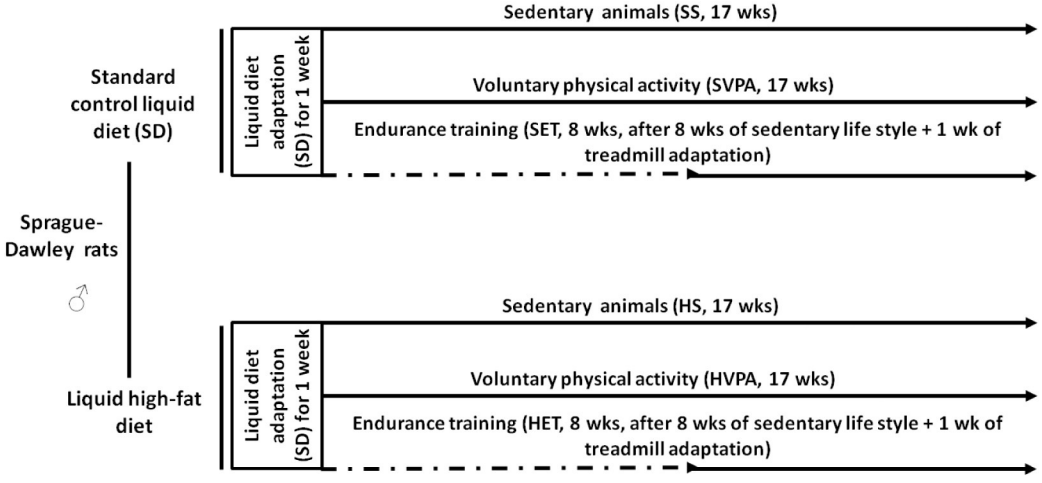

Fig. 1. Design of the diet and exercise training protocol. HET, endurance trained animals with access to high-fat liquid diet; HS, sedentary animals with access to high-fat liquid diet; HVPA, voluntarily physically active animals with access to high-fat liquid diet; SD, standard control liquid diet; SET, endurance trained animals with access to standard control liquid diet; SS, sedentary animals with access to standard control liquid diet; SVPA, voluntarily physically active animals with access to standard control liquid diet; wk(s), week(s).